

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Entomology Papers from Other Sources

Entomology Collections, Miscellaneous

1970

Suppression and Elimination of an Island Population of *Culex pipiens quinquefasciatus* with Sterile Males

R. S. Patterson
ARS-USDA

D. E. Weidhaas
ARS-USDA

H. R. Ford
ARS-USDA

C. S. Lofgren
ARS-USDA

Follow this and additional works at: <https://digitalcommons.unl.edu/entomologyother>

 Part of the [Entomology Commons](#)

Patterson, R. S.; Weidhaas, D. E.; Ford, H. R.; and Lofgren, C. S., "Suppression and Elimination of an Island Population of *Culex pipiens quinquefasciatus* with Sterile Males" (1970). *Entomology Papers from Other Sources*. 50.

<https://digitalcommons.unl.edu/entomologyother/50>

This Article is brought to you for free and open access by the Entomology Collections, Miscellaneous at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Entomology Papers from Other Sources by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

minutes and reached levels comparable to those obtained in our laboratory following thyroidectomy. The time course of this response is similar to that which is observed following administration of hypothalamic extracts with TSH-releasing activity (unpublished results).

Control injections of 0.9 percent NaCl, crude extracts of porcine cerebral cortex (equivalent in weight to five stalk median eminences), synthetic mast cell releaser 48/80 (0.03 to 0.5 mg), histamine phosphate (0.25 mg), and Pitressin (0.5 unit) were all without effect on plasma TSH levels.

These experiments demonstrate for the first time the time course of TSH release in the rat following hypothalamic electrical stimulation. The increases in plasma TSH levels were rapid and corresponded closely to those reported for growth hormone following stimulation of the ventromedial hypothalamic nucleus in the rat (8). Since peripheral blood was sampled in these experiments, the quantity of TSH secreted must be considerable in order to elevate blood levels two- to fivefold. It is hypothesized that release of TSH from the pituitary is dependent upon a thyrotropin-releasing factor synthesized in the neurons of the basal hypothalamus. The rapid responses which occur following electrical excitation of this region are suggestive of release of preformed TRF from these cells. The administration of "synthetic" TRF in the present experiments produced a more rapid increase in plasma TSH levels, which reached thyroidectomy levels (perhaps a maximal re-

sponse) within 5 minutes. It is probable that the earlier response following a single injection of TRF reflects the difference between a sudden stimulus and a more sustained release of smaller quantities of TRF during the period of hypothalamic stimulation.

JOSEPH B. MARTIN*

Department of Medical and Pediatric Specialties, University of Connecticut School of Medicine, Farmington, and Department of Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, New York

SEYMOUR REICHLIN

Department of Medical and Pediatric Specialties, University of Connecticut School of Medicine, Farmington

References and Notes

1. G. W. Harris and J. W. Woods, *J. Physiol. (London)* **143**, 246 (1958); H. J. Campbell, R. George, G. W. Harris, *ibid.* **152**, 527 (1960); K. Shizume, K. Matsuda, M. Irie, S. Iino, J. Ishii, S. Nagataki, F. Matsuzaki, S. Okinaka, *Endocrinology* **70**, 298 (1962); W. C. Boop and J. Story, *Neurology* **16**, 1167 (1966); M. Vertes, Z. Vertes, S. Kovacs, *Acta Physiol. Acad. Sci. Hung.* **27**, 229 (1965); R. L. W. Averill and T. H. Kennedy, *N.Z. Med. J.* **65**, 398 (1966).
2. S. A. D'Angelo and J. Snyder, *Endocrinology* **73**, 75 (1963).
3. — and J. M. Grodin, *ibid.* **75**, 417 (1964); R. L. W. Averill and D. F. Salaman, *ibid.* **81**, 173 (1967).
4. S. Reichlin, J. B. Martin, R. L. Boshans, D. S. Schalch, J. G. Pierce, J. Bollinger, in preparation.
5. R. Burgus, T. F. Dunn, D. Desiderio, W. Vale, R. Guillemin, C. R. Hebd., *Seances Acad. Sci. Paris* **269**, 226 (1969).
6. J. Bøler, F. Enzmann, K. Folkers, C. Y. Bowers, A. V. Schally, *Biochem. Biophys. Res. Commun.* **37**, 705 (1969).
7. J. de Groot, *J. Comp. Neurol.* **113**, 389 (1959).
8. L. A. Frohman, L. L. Bernardis, K. J. Kant, *Science* **162**, 580 (1968).
9. We thank Drs. Roger Guillemin and Andrew V. Schally who have allowed us to read their manuscripts in advance of publication. Supported by PHS grant AM 13695-01.

* Centennial Fellow, Medical Research Council of Canada.

9 February 1970

males were competitive with the wild males, and, when the test was discontinued after 8 weeks because of the onset of cool weather, 85 percent of the egg rafts collected were sterile (1).

We conducted a similar study in 1969 to show that sterile males could be used to suppress or eliminate an isolated population of *C. p. quinquefasciatus*. The test site was Seahorse Key, a crescent-shaped island, about 1.6 by 0.2 km; it is covered by dense woods except for a large T-shaped clearing that extends across the center of the island.

The primary larval breeding site for *C. p. quinquefasciatus* was a partially open septic tank that received domestic wastes. Breeding also occurred in containers that had been discarded in the woods where they collected rainwater.

The mosquitoes were reared as larvae at the Insects Affecting Man and Animals Research Laboratory at Gainesville, Florida. We sterilized the mosquitoes in the pupal stage by exposing them for 4 hours to an aqueous solution of 0.75 percent thiotepa [tris(1-aziridinyl)phosphine sulfide]. The mosquitoes were then placed on wet blotting paper in pans in Styrofoam boxes chilled with ice and transported to the release site. At Seahorse Key, the pans were placed under the laboratory, the lids were removed, and fresh water was added to float the remaining pupae. The sterile adults dispersed rapidly into the environment, and large numbers were seen in mating swarms at dusk and dawn throughout the cleared area of the island.

Egg rafts were collected from the indigenous population in 12 ovitraps (40-liter tubs containing larval food) located at the edge of the woods and in open areas near the main breeding sites. The egg rafts were collected and counted daily and then placed in separate 18.5-ml vials containing 5 ml of water. Most fertile eggs hatched within 24 hours, but all eggs were kept for 4 days before they were determined to be viable or non-viable. Since the larvae from fertile egg rafts were destroyed, our assay procedures exerted an additional control on the mosquito population similar to what could be achieved with larvicides or removal of breeding sites.

Because fluctuations in egg production caused by varying weather conditions made the daily records difficult to interpret, we combined the data for each

Suppression and Elimination of an Island Population of *Culex pipiens quinquefasciatus* with Sterile Males

Abstract. *The release of 8,400 to 18,000 males per day of Culex pipiens quinquefasciatus Say which had been exposed to a sterilizing agent (thiotepa), suppressed and eliminated an indigenous population of this mosquito on an island off the coast of Florida in a 10-week period. Sterile males were effective in seeking out and mating with females on the island.*

Culex pipiens quinquefasciatus Say (= *Culex fatigans* Wiedemann) is a vector of human diseases, particularly filariasis, and can be controlled with insecticides. We now report that the technique of male sterilization can be used to suppress population build-ups of *C. p.*

quinquefasciatus or to eliminate it from certain areas. We studied methods of sterilization and mass rearing and the biology of this species. In 1968 we released males, sterilized chemically with tepa, on Seahorse Key, located about 3.2 km off the west coast of Florida. These

Table 1. Release, sterility, and control data for the sterile male release experiment with *C. p. quinquefasciatus* on Seahorse Key. Our ovitraps collected about 50 percent of the total number of egg rafts that were oviposited by females each day. These egg rafts were destroyed.

Generation	Release data		Egg raft data				Reduction in No. of egg rafts collected (%)	
	Sterile males released (No./day)	Sterile: normal males	Laid in ovitraps (No./day)	Expected to be sterile (%)	Actually sterile (%)	Sterile or destroyed (%)	Total	Fertile
1	0		228		0	50		
2	8,400	3:1	146	75	62	81	36	75
3	13,000	4:1	151	80	85	92	34	90
4	11,000	12:1	47	92	82	91	79	96
5	18,000	100:1	9	99	84	92	96	99.4
6	16,000	100:1	8	99	95	97.5	96	99.8

generation (2 weeks) and calculated a daily average for this period. The release, sterility, and control data for the six generations during the test (one generation before and five generations during the release of males sterilized by thiotepa) were recorded (Table 1).

We released an average of 8,400 to 18,000 sterile males per day during the second through the sixth generation (10 June to 18 August 1969). While we could not definitely determine the ratio of sterile to normal males, we were able to make fairly accurate estimates from our information on the size of the indigenous female population. About 10 percent of the females that emerge as adults survive and lay eggs (2); therefore, the number of egg rafts laid per day multiplied by ten approximates the number of females emerging into the population each day. Since males and females occur in a ratio of about 1:1, the same number of males emerged each day. Because we could not collect all the egg rafts from the natural breeding sites, it was necessary to determine what percentage of the total egg rafts laid each day were oviposited in the ovitraps. This was done by releasing females tagged with ^{32}P by exposure of the larvae to the radioactive material in water solution and determining the difference in the number of radioactive eggs laid in the ovitraps when all known breeding sites were either covered or uncovered (the test was completed after the releases had stopped). Five percent of the total number of tagged females laid their eggs in the ovitraps when the natural breeding sites were uncovered, compared with 10 percent when they were covered. Therefore, the ovitraps were 50 percent efficient and only 10 percent of the females emerging into a population survive and lay eggs. Also, the ratio of sterile to normal males during the period of releases ranged from

3:1 in the second generation to 100:1 in the fifth and sixth generations.

Theoretically, the ratio of sterile to normal males should have produced from 67 percent to as much as 99 percent sterility of the females by the end of the experiment. Actually, the percentage of sterile egg rafts remained below that expected in all generations except the third. We believe that except in the second generation (at which time some fertile females that had mated prior to our release of sterile males still remained in the population) this was caused by immigration of fertilized females onto the island from fishing boats or from the mainland, because studies in large outdoor cages had demonstrated that males sterilized with thiotepa are competitive with normal males.

The *C. p. quinquefasciatus* females can migrate more than 3.2 km over land or water (3). Also, gravid females are usually present in the cabins of fishing boats that often anchor near the island at night. Moreover, if immigration of fertile females did occur it would be most noticeable in a declining population like the one we had during the final 4 weeks of our test when we obtained complete sterility of all eggs on 19 of the 28 days. Also, we invariably obtained fertile egg rafts on the days after nights when fishing boats were anchored near the island. Extensive larval surveys conducted during the last 2 weeks revealed no *C. p. quinquefasciatus* larvae in any indigenous breeding sites.

In assessing the total effect of our procedures, we must also include the effect of removal of egg rafts. Since our ovitraps collected about 50 percent of the total number of egg rafts laid each day, we used this factor to determine that the total reduction in reproductive capacity as a result of sterilization and egg removal increased from 81 percent

in the second generation to 97.5 percent in the sixth generation.

During the 10 weeks that we released sterile males, the number of egg rafts collected per day dropped from a count before treatment of 228 to 8 in the ninth and tenth weeks—a reduction of 96 percent in the population. Moreover, during the sixth generation, 95 percent of the egg rafts were sterile. Thus, the actual overall reduction in fertile egg rafts was 99.4 and 99.8 percent in the last two generations.

Since the island was not completely isolated, we did not expect to eradicate the population of *C. p. quinquefasciatus* permanently. However, because no larvae were found during the last 2 weeks in any of the natural larval breeding sites, and because we obtained a significant suppression of the adult population (as indicated by a 96 percent decrease in egg rafts laid), we feel that we could have achieved eradication if the island had been completely isolated. Thus sterile males can be used to suppress and eradicate some species of mosquitoes under isolated conditions. However, more research is needed before such eradication can be accomplished over large areas. Migration patterns of wild and sterile males, rates of increase in populations at different times of the year, and total population densities need to be investigated (4). Obviously other population suppressants such as insecticides, reduction in breeding sources, and biological control will have to be used to decrease a total population in a large area to a level commensurate with the mass rearing capabilities (5).

R. S. PATTERSON, D. E. WEIDHAAS
H. R. FORD, C. S. LOFGREN
United States Department of
Agriculture, Agricultural Research
Service, Entomology Research Division,
Gainesville, Florida 32601

References and Notes

1. R. S. Patterson, H. R. Ford, C. S. Lofgren, D. E. Weidhaas, *Mosquito News* 30(1), 21 (1970).
2. R. S. Patterson, unpublished data.
3. E. M. Fussell, *Mosquito News* 24, 422 (1964); A. W. Linquist, T. Ikeshoji, B. Grab, B. De Meillon, B. H. Khan, *World Health Organ. Bull.* 36, 21 (1967).
4. More comprehensive data on rates of increase and population densities noted in this study will be published elsewhere. These data indicate that more than 90 percent sterility was required before the natural rate of increase of this mosquito population was exceeded.
5. Rearing and sterilization of mosquitoes in this experiment required about 6 man-hours per day. The *C. p. quinquefasciatus* is easy to rear and we foresee no difficulty in producing very large numbers of mosquitoes.
6. We thank Dr. B. Smittle for technical assistance in the radioactive tagging study, Mr. M. Boston for supervising the rearing of the mosquitoes, and Dr. G. B. White [*Nature* 210, 1372 (1966)] for suggesting the technique of sterilizing pupae with thiotepa.

23 January 1970; revised 13 March 1970 ■

Intracranial Self-Stimulation and Wakefulness: Effect of Manipulating Ambient Brain Catecholamines

Abstract. Rats were given disulfiram, an inhibitor of norepinephrine biosynthesis, to see if norepinephrine is a transmitter for motivation in electrical stimulation of the brain. Animals given the drug paused in bar pressing, appearing asleep or sedated; if replaced on the bar, they always resumed pressing at normal rates. The decrease in bar pressing may result from a direct or indirect effect of the drug on wakefulness rather than on reward.

One can study the neurohumoral basis of integrative pathways in the central nervous system by observing drug-induced changes in behavior established and maintained by electrical stimulation of the brain (ESB) as the motivating stimulus. Wise and Stein (1) reported that the rate of bar pressing for rewarding brain stimulation was markedly reduced in rats treated with disulfiram, a potent inhibitor of norepinephrine (NE) synthesis. Assuming that magnitude of reward is correlated directly with rate, they inferred that NE was the neurotransmitter for reward pathways in intracranial self-stimulation (ICS) because diminution of NE led to diminution of rate. Using the work of Wise and Stein (1) and the theoretical position developed by Deutsch (2) as foundations, this laboratory tried to establish which of the two pathways in ICS (drive or reward) used catecholamines as neurotransmitters. Initially, I tried to confirm Wise and Stein's (1) conclusions. Tygon-insulated stainless steel monopolar electrodes were stereotaxically placed and permanently implanted in the medial forebrain bundle (MFB) [coordinates $A = 5.8$, $V = 2.25$, $L = 1.75$ (3)] of four adult male Simonsen Sprague-Dawley rats (350–450 g). The animals were then trained to stimulate themselves by pressing a bar in a modified Skinner apparatus in 1-hour daily practice sessions until stable performance was observed. Suitable current intensities were determined for each animal and chosen as the minimum which would sustain bar pressing. The stimulating apparatus was the same used by

Deutsch (4), with the duration of pulse trains lasting either 0.15 or 0.8 second. The duration was varied only to permit more sensitive measurement of rate variation, but results were essentially the same for both durations. Disulfiram (100 mg/kg) (5), suspended in 1 ml of physiological saline with 0.03 percent Tween-80, was administered intraperitoneally. Because of its viscosity the suspension was administered either by syringe or with the aid of silicone tubes permanently implanted in the peritoneal cavity (6). Results were recorded by means of an event recorder and a digital counter.

Base-line rates of bar pressing for all animals were obtained from one or more sessions consisting of a maximum of 1½ hours of bar pressing per session. Rates were calculated every 5 minutes during each session. All base-line data obtained were used. Animals to be given disulfiram were run once for five continuous hours and injected at the end of the first hour; all data after the onset of observable symptoms were used, but no data were used if the animal did not evince the pharmacological symptoms of NE inhibition.

The onset of drug effect was defined for purposes of data collection as the first pause in the animal's responding which exceeded the longest pause recorded during base line. During base-line recording, if a rat paused he voluntarily resumed bar pressing. After administration of the drug rats pausing longer than the maximum base-line pause never voluntarily resumed bar pressing; therefore we returned them to the bar according

to an arbitrary criterion. Rats were permitted a pause of at least base-line maximum plus 5 seconds. For example, an animal which paused no longer than 60 seconds under control conditions would be allowed a pause of 65 seconds after treatment with the drug. This procedure differs from that of Wise and Stein (1), who sometimes handled the animal but did not return it to the bar. The procedure was introduced because it was suspected that the stops in bar pressing were due to the sedation reported by Wise and Stein (1), since the drugged animals were always found asleep during pauses. If the animals had gone to sleep because of diminishing returns from ICS, then replacing them on the bar should not have caused them to resume bar pressing. If, on the other hand, the animal had gone to sleep because of sedation and drowsiness, and stopped pressing the bar as a secondary result, then replacing it on the bar and so awakening it should have produced normal rates of bar pressing. Furthermore, even if the sedation and drowsiness were a result of drug toxicity or pain from peritoneal irritation, rather than a result of an effect on central NE pathways, it would still seem that the drug has no effect on reward that can be measured in Wise and Stein's (1) experimental design. The animals, however, seemed to show no discomfort at the time of the injection, and the symptoms they did evince were similar to those described for side effects of drugs antagonistic to NE (7).

Rates were thus measured to see whether Wise and Stein's (1) results with disulfiram could be obtained under our conditions. The numbers of criterion pauses for each condition were also compared. We expected that if reward were being diminished by NE inhibition, the response rate would be consistently depressed by the drug; if an animal paused in responding, it should not resume pressing the bar, either voluntarily or after being replaced on the bar. This particular observation is not reported by Wise and Stein (1); however, since measurement of rate is confounded by the rats' sedation and sleepiness in our study, as in Wise and Stein's (1), forcing the animal to resume bar pressing by replacement is a necessary control in order for one to determine completely the value of ESB to a drowsy animal. Resumption of bar pressing at a high rate after replacement on the bar would indicate that the animal does not find the stimulation less motivating than be-